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Sex determination and the evolution of dioecy from monoecy in *Sagittaria latifolia* (Alismataceae)

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The role of mutations of small versus large effect in adaptive evolution is of considerable interest to evolutionary biologists. The major evolutionary pathways for the origin of dioecy in plants (the gynodioecy and monoecy–paradioecy pathways) are often distinguished by the number of mutations involved and the magnitude of their effects. Here, we investigate the genetic and environmental determinants of sex in *Sagittaria latifolia*, a species with both monoecious and dioecious populations, and evaluate evidence for the evolution of dioecy via gynodioecy or monoecy–paradioecy. We crossed plants of the two sexual systems to generate F_1, F_2 and backcross progeny, and grew clones from dioecious populations in low- and high-fertilizer conditions to examine sex inconstancy in females and males. Several lines of evidence implicate two-locus control of the sex phenotypes. In dioecious populations sex is determined by Mendelian segregation of alleles, with males heterozygous at both the male- and female-sterility loci. In monoecious populations, plants are homozygous for alleles dominant to male sterility in females and recessive to female sterility in males. Experimental manipulation of resources revealed sex inconstancy in males but not females. These results are consistent with predictions for the evolution of dioecy via gynodioecy, rather than the expected monoecy–paradioecy pathway, given the ancestral monoecious condition.

Keywords: evolution of dioecy; genetics of sex; monoecy; sex inconstancy; sterility mutations

1. INTRODUCTION

Dioecy (separate sexes) has evolved independently from cosexuality in nearly half of all angiosperm families through a variety of selective forces and genetic mechanisms (reviewed in Geber *et al.* 1999). Two primary evolutionary pathways are commonly distinguished—the gynodioecy pathway, in which the intermediate stage involves populations polymorphic for females and hermaphrodites (Lloyd 1976), and the monoecy–paradioecy pathway, in which transitional populations are bimodal for gender and exhibit quantitative variation in female and male fertility (Lloyd 1980). These two pathways therefore differ in the relative importance of genes with large versus small phenotypic effects. The frequency with which each pathway has led to the evolution of dioecy is controversial: two recent comparative studies have come to contrasting conclusions (Renner & Ricklefs 1995; Weiblen *et al.* 2000).

Distinguishing between the evolutionary pathways for the origins of dioecy requires detailed studies of the genetic and environmental factors governing sex expression. First, the gynodioecy pathway entails mutations of large effect, and may often involve as few as two mutations, one determining male sterility and the other female sterility (B. Charlesworth & D. Charlesworth 1978). By contrast, the monoecy–paradioecy pathway is generally thought to involve gradual changes in quantitative variation in sex allocation via many mutational steps (D. Charlesworth & B. Charlesworth 1978). Second, for the gynodioecy pathway, male-sterility mutations of large effect usually prevent the production of pollen in females, and therefore sex inconstancy (e.g. the production of ovules by male plants) commonly occurs only among male plants (Lloyd 1980). By contrast, because the monoecy–paradioecy pathway involves multiple mutations, possibly in combination with environmental regulation of sex expression, sex inconstancy is a characteristic feature of both female and male plants (Lloyd 1975, 1980; Freeman *et al.* 1997; Renner & Won 2001). Investigations of the genetics of sex determination and patterns of sex inconstancy between the sexes should enable discrimination between the two main pathways involved in the origin of dioecy.

Studies of sex determination in plants have revealed a variety of genetic systems, including sex chromosomes and cytoplasmic factors (Grant 1999; Charlesworth 2002); however, in most cases males are heterozygous at sex-determining loci (or heterogametic when sex chromosomes occur; Westergaard 1958). Based on this observation, Charlesworth & Guttman (1999) developed an explicit genetic model for the evolution of dioecy via the gynodioecy pathway involving simple Mendelian inheritance and two sex-determining loci (figure 1). First, a cosexual population is invaded by a recessive male-sterility mutation leading to the establishment of females. This is then followed by the spread of a dominant suppressor of female fertility among cosexuals, conferring male heterozygosity. A necessary condition for the evolution of sex-determining loci is that the male- and female-sterility loci are linked, reducing the occurrence of recombination between these loci (Charlesworth & Guttman 1999). Although this pathway is usually envisioned for populations with hermaphrodite flowers (see discussion in Renner & Won (2001)), we know of no *a priori* reason why these genetic mechanisms could not also be involved in the evolution of dioecy from monoecy. If so, the common assumption that dioecy originates via the monoecy–paradioecy pathway in monoecious groups may not always be true.

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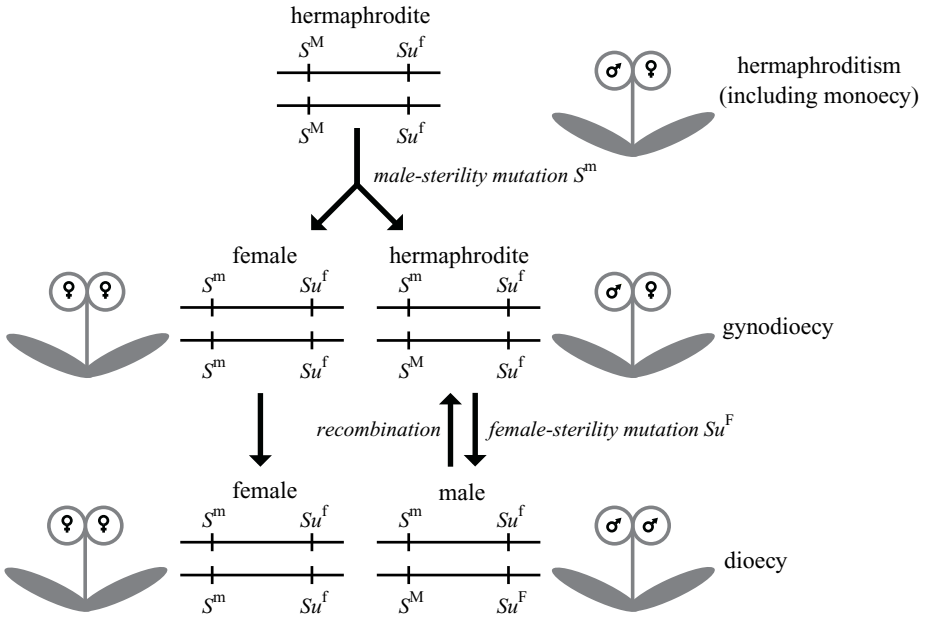

Figure 1. Genetic changes inferred in the evolutionary transition from hermaphroditism to dioecy via the gynodioecy pathway in *Sagittaria latifolia*, following the genetic model of Charlesworth & Guttman (1999).

Here, we investigate the genetics of sex determination and patterns of sex inconstancy in *Sagittaria latifolia* Willd. (Alismataceae). This was possible because *S. latifolia* is unusual in possessing fully interfertile monoecious and dioecious populations (Dorken *et al.* 2002; Dorken & Barrett 2003). Our primary goal in this study was to determine which pathway was involved in the evolution of dioecy in *S. latifolia*. Simple Mendelian inheritance of sex combined with sex inconstancy in males but not females would support the gynodioecy pathway. By contrast, evidence for many loci governing sex expression, and sex inconstancy in both females and males would be more consistent with the monoecy–paradioecy pathway.

2. MATERIAL AND METHODS

(a) *Sampling and crossing programme*

Sagittaria latifolia is a clonal aquatic common to a variety of wetland habitats throughout eastern North America (Dorken & Barrett 2003). In 1999, we randomly collected plants from monoecious and dioecious populations in southern Ontario, Canada, and grew them under common glasshouse conditions for 1 year (see Dorken & Barrett (2003) for details). In 2000 we selected plants from two dioecious populations that differed in the presence versus absence of sex inconstancy, and four monoecious populations to act as parents in the crossing programme. We used eight and six plants from the dioecious and monoecious populations, respectively, as F_1 parents. Out of these plants, we used three females and one inconstant male as maternal parents, four males plus the inconstant male as paternal parents, and the remaining plants as both maternal and paternal parents.

We used hand crosses to generate 18 F_1 seed families in a pollinator-free glasshouse at the University of Toronto. We performed crosses by removing freshly opened male flowers and rubbing them against receptive female flowers. Female flowers were tagged and fruits were collected at maturity. We dried the fruits at room temperature for 3 days and stored them at 5 °C for four months. Three weeks prior to sowing, we placed the seed families into vials filled with water and maintained them at 5 °C. After sowing into pots containing sandy loam the seed families were flooded with water containing No-Damp. Following germination, plants were grown in 10.2 cm pots that were randomly arranged in water-filled trays and fertilized regularly. We screened plants at three allozyme loci to verify the parentage of the progeny.

To generate F_2 progeny, we performed hand crosses between full- and half-sibs from the F_1 generation. We also generated backcross progeny by crossing F_1 plants with individuals from the parental generation. We used 12 plants as maternal parents and 25 plants as paternal parents for a total of 23 F_2 and backcross families. All populations were represented in the F_1 and F_2 families. For all progeny in the F_1 and F_2 generations, we scored the presence of female and male flowers on at least one inflorescence. As sex expression is size dependent in *S. latifolia* (Sarkissian *et al.* 2001), plants that produced only male flowers were monitored for the entire growing season to ensure that they were indeed male. Plants that produced only two inflorescences were re-grown from corms the following year to verify their sex. Segregation of sex phenotypes in progeny arrays was tested against Mendelian expectations using replicated goodness-of-fit tests (i.e. *G*-tests; Sokal & Rohlf 1995) to examine heterogeneity among crosses.

(b) *Patterns of sex inconstancy*

To investigate sex inconstancy in females and males from dioecious populations we grew replicates of clonally propagated corms under high- and low-fertilizer treatments. The plants used in this experiment came from five dioecious populations, including the two dioecious populations mentioned in § 2a. For this experiment, we collected 10 plants from each dioecious population, giving a total of 27 female and 23 male plants. We grew plants under common glasshouse conditions for an entire growing season, at the end of which we collected the four largest corms from each plant ($n = 200$) and stored them in a cold room for five months. The following April, we planted corms into 15.2 cm pots, placed each pot in a separate 5 l bucket and assigned two ramets from each clonal genotype to the high-fertilizer treatment (weekly fertilization with 200 ml of a 10%

Table 1. Segregation of female, male and hermaphrodite plants of *Sagittaria latifolia* in the F_1 progeny of crosses between (*a*) females (f) and males (m) from dioecious populations, and (*b*) males from dioecious populations and hermaphrodites (h) from monoecious populations.
(For each cross type, we indicate the expected frequency of each sex in the progeny according to the model of sex determination described by Charlesworth & Guttman (1999). For each cross, the sex of the paternal and maternal parents are indicated in parentheses, followed by the putative genotypes of the parents.)

cross	putative genotypes	male	female	*n*	*G*
(*a*) predicted ratios: 1 : 1 male : female					
1 (m × f)	$Su^FS^M/Su^fS^m \times Su^fS^m/Su^fS^m$	0.57	0.43	35	0.72
2 (m × f)	$Su^FS^M/Su^fS^m \times Su^fS^m/Su^fS^m$	0.48	0.52	33	0.03
3 (m × f)	$Su^FS^M/Su^fS^m \times Su^fS^m/Su^fS^m$	0.42	0.58	134	3.62
total		0.46	0.54	202	
				d.f.	*p*
		G_{het}	2.77	2	0.25
		G_{pooled}	1.61	1	0.21
(*b*) predicted ratios: 1 : 1 male : hermaphrodite		male	hermaphrodite	*n*	*G*
4 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.38	0.62	39	2.10
6 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.40	0.60	40	1.61
9 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.46	0.54	41	0.22
12 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.48	0.52	42	0.10
17 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.32	0.68	31	3.99[a]
20 (m × h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^M$	0.44	0.56	48	0.75
total		0.42	0.58	241	
				d.f.	*p*
		G_{het}	2.40	5	0.79
		G_{pooled}	6.34[a]	1	0.01

[a] Values of *G* corresponding to $p < 0.05$.

20 : 20 : 20 N : P : K fertilizer solution) and two to the low-fertilizer treatment (no fertilizer following transplantation). We monitored flower production daily over the entire growing season and counted the number of female and male flowers per inflorescence.

3. RESULTS

(a) *Simple Mendelian control of sex*

Crosses between females and males resulted in 1 : 1 progeny ratios (table 1*a*). All progeny from crosses between hermaphrodites from monoecious populations and females from dioecious populations were hermaphroditic (four families of monoecious hermaphrodites × females; total $n = 141$ progeny). Full-sib crosses between F_1 progenies and backcrosses confirmed that males from dioecious populations are heterozygous for loci governing male and female sterility. Plants from monoecious populations are homozygous for alleles dominant to sex-determining alleles in females, and recessive to those in males (table 2). We found no heterogeneity in ratios among crosses with the same expected distribution of sex phenotypes among the progeny (tables 1 and 2).

Crosses with the inconstant male revealed that sex inconstancy in dioecious populations is also governed by the segregation of Mendelian alleles, with inconstant males heterozygous for male, but not female, sterility. For the F_1 generation, when crossed to a male, the progeny conformed to a 2 : 1 : 1 male : hermaphrodite : female distribution (57% male, 25% hermaphrodite, 18% female, $n = 126$ progeny, $G = 3.77$; family 15). When crossed to a female, the distribution of sex phenotypes conformed to a 1 : 1 hermaphrodite : female distribution (59% hermaphrodite, 41% female, $n = 34$ progeny, $G = 1.06$; family 16). Finally, all the progeny from two crosses with plants from monoecious populations were hermaphroditic ($n = 99$ progeny; families 13 and 14). For the F_2 generation, the distribution of sex phenotypes among progeny arrays was also consistent with inconstant males being heterozygous for male but not female sterility (table 2, crosses D, G, H, I, L and R).

There were two significant departures from the Mendelian expectations of the genetic model of Charlesworth & Guttman (1999). The first involved progeny of an inconstant male that was selfed. If inconstant males are heterozygous at the male-sterility locus, we would expect a 3 : 1 ratio of inconstant males to females. However, all progeny from this self-fertilization were hermaphroditic ($n = 58$). This result was unexpected because the progeny of crosses between this inconstant male and female and male plants were not significantly different from 1 : 1 hermaphrodite : female and 2 : 1 : 1 male : hermaphrodite : female expectations, respectively (see above). Female progeny were also absent from three full-sib crosses between the

Table 2. Segregation of female, male and hermaphrodite plants of *Sagittaria latifolia* in the F_2 and backcross progeny of crosses between: (*a*) full-sib F_1 males (m) and hermaphrodites (h), and parental-generation males (Pm) and F_1 hermaphrodites; (*b*) full- and half-sib F_1 hermaphrodites; (*c*) full- and half-sib F_1 males and hermaphrodites, and F_1 males and parental-generation females (Pf), and (*d*) F_1 hermaphrodites and parental-generation females.
(For each cross type, we indicate the expected frequency of each sex in the progeny according to the model of sex determination described by Charlesworth & Guttman (1999). For each cross, we indicate the paternal parent, followed by the maternal parent, with the F_1 family to which each plant belonged, in parentheses, followed by the putative genotypes of the parents.)

cross	putative genotypes	male	hermaphrodite	female	*n*	*G*
(*a*) predicted ratios: 2 : 1 : 1 male : hermaphrodite : female						
A (Pm × 6h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.43	0.22	0.35	72	3.39
B (Pm × 11h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.49	0.26	0.25	121	0.02
C (Pm × 12h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.42	0.25	0.33	98	4.04
D (Pm × 13h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.50	0.20	0.30	71	1.33
E (Pm × 18h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.55	0.24	0.21	80	0.91
F (Pm × 18h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.45	0.31	0.24	74	1.70
G (15m × 15h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.49	0.30	0.21	80	1.25
H (15m × 15h)	$Su^FS^M/Su^fS^m \times Su^fS^M/Su^fS^m$	0.50	0.29	0.21	80	0.90
total		0.48	0.26	0.26	676	
					d.f.	*p*
			G_{het}	12.22	14	0.59
			G_{pooled}	1.37	2	0.63
(*b*) predicted ratios: 3 : 1 hermaphrodite : female			hermaphrodite	female	*n*	*G*
I (15h × 16h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.69	0.31	120	2.34
J (11h × 11h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.79	0.21	95	0.69
K (18h × 18h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.70	0.30	87	1.06
L (15h × 18h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.77	0.23	95	0.28
M (6h × 6h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.81	0.19	47	0.91
N (11h × 12h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.72	0.28	46	0.17
O (12h × 12h)	$Su^fS^M/Su^fS^m \times Su^fS^M/Su^fS^m$		0.60	0.40	65	7.03[a]
total			0.73	0.27	555	
					d.f.	*p*
			G_{het}	10.62	6	0.10
			G_{pooled}	2.41	1	0.12
(*c*) predicted ratios: 1 : 1 male : hermaphrodite		male	hermaphrodite		*n*	*G*
P (6m × 6h)	$Su^FS^M/Su^fS^M \times Su^fS^M/Su^fS^m$	0.47	0.53		45	0.56
Q (18m × 18h)	$Su^FS^M/Su^fS^M \times Su^fS^M/Su^fS^m$	0.53	0.47		85	0.29
R (18m × 15h)	$Su^FS^M/Su^fS^M \times Su^fS^M/Su^fS^m$	0.58	0.42		57	1.43
S (12m × 12h)	$Su^FS^M/Su^fS^M \times Su^fS^M/Su^fS^m$	0.55	0.45		51	0.49
T (12m × 12h)	$Su^FS^M/Su^fS^M \times Su^fS^M/Su^fS^m$	0.61	0.39		71	3.19
U (17m × Pf)	$Su^FS^M/Su^fS^M \times Su^fS^m/Su^fS^m$	0.48	0.52		115	0.22
total		0.53	0.47		424	
					d.f.	*p*
			G_{het}	4.24	5	0.52
			G_{pooled}	1.82	1	0.18
(*d*) predicted ratios: 1 : 1 hermaphrodite : female			hermaphrodite	female	*n*	*G*
V (11h × Pf)	$Su^fS^M/Su^fS^m \times Su^fS^m/Su^fS^m$		0.43	0.57	173	3.07
W (18h × Pf)	$Su^fS^M/Su^fS^m \times Su^fS^m/Su^fS^m$		0.58	0.42	31	0.81
total			0.46	0.54	204	
					d.f.	*p*
			G_{het}	2.29	1	0.13
			G_{pooled}	1.59	1	0.21

[a] Values of *G* corresponding to $p < 0.05$; however, these values are not significant when the table-wide α is adjusted for the number of tests performed.

F_1 progeny of the inconstant male and a plant from a monoecious population ($n = 160$ progeny). In these F_2 families, all progeny were hermaphroditic.

The second departure from model expectations was in crosses between males from dioecious populations and monoecious plants in the F_1 generation (table 1*b*). For these progeny, more hermaphrodites than males were recovered, contrasting with the 1 : 1 expectation for this cross. However, when F_1 males were used as parents, the progeny of these crosses always conformed to the Mendelian expectations of the model (table 2*c*). Indeed, for the pooled data all F_2 and backcross progeny conformed to model expectations (table 2).

(b) *Sex inconstancy in males but not females*

In dioecious populations we detected considerable sex inconstancy in male plants (26% of 92 ramets), but not female plants. Under high-fertilizer conditions 10 out of 23 male genotypes produced some combination of male and female flowers. However, most of these male genotypes produced only male flowers when grown under low-fertilizer conditions (21 out of 23 clonal genotypes). No female plants produced male flowers regardless of growing conditions, although not all females flowered in the low-fertilizer treatment.

4. DISCUSSION

Our study demonstrates simple inheritance of sex in *S. latifolia* and that sex inconstancy is restricted to male plants. These results are consistent with predictions for the evolution of dioecy via the gynodioecy pathway, rather than the monoecy–paradioecy pathway. They cast doubt on the common assumption that if monoecious and dioecious populations co-occur among related taxa, sexual dimorphism evolved via paradioecy. In § 4a we discuss these findings, which indicate that the results of attempts to distinguish between evolutionary pathways for the evolution of dioecy may often be misleading without a detailed understanding of the genetic and environmental determinants of sex.

(a) *Sex determination in* Sagittaria latifolia

Sex in *S. latifolia* is determined by the Mendelian segregation of alleles, yielding a limited number of genotypes. In dioecious populations males are heterozygous at loci governing male and female sterility. In monoecious populations, plants are homozygous for alleles that are dominant to male-sterility alleles in females, and recessive to alleles suppressing female fertility in males. Using the notation of Charlesworth & Guttman (1999; figure 1), in natural populations males would have the genotype $Su^{F}S^{M}/Su^{f}S^{m}$ (where *Su* and *S* are linked loci, the first governing female fertility and the second male fertility), females are $Su^{f}S^{m}/Su^{f}S^{m}$, while plants from monoecious populations are $Su^{f}S^{M}/Su^{f}S^{M}$. When crosses are performed between the sexual systems two additional genotypes arise (one male and one hermaphrodite; table 2), yielding a total of five genotypes governing the occurrence of three sex phenotypes. Progeny ratios similar to ours were reported by Galán (1951) from crosses between monoecious and dioecious populations of *Ecballium elaterium* (Cucurbitaceae). He interpreted these ratios as resulting from a one-locus three-allele model of sex determination (see Grant 1999). This raises the question of how many loci are involved in sex determination in *S. latifolia*.

In our study, the absence of recombinant progeny (e.g. hermaphrodites in the progeny of male × female crosses) could indicate that one locus is involved in sex determination in *S. latifolia*. However, as pointed out by Westergaard (1958) and Charlesworth & Guttman (1999), it is highly unlikely that the genetic changes required for the evolution of dioecy would occur at a single locus. The absence of recombinant progeny may simply indicate that the sex-determining loci are tightly linked (see Charlesworth 2002) and therefore larger sample sizes than were used here would be required to detect recombination.

Two lines of evidence support a two-locus model of sex determination in *S. latifolia*. As inconstant males are heterozygous for alleles conferring male sterility, with one locus, sex inconstancy could only result from either gene flow from monoecious populations or maintenance of ancestral 'hermaphrodite alleles' in dioecious populations. Our studies of natural populations do not support either of these alternatives. First, sex inconstancy is common in regions where monoecious populations are absent. Moreover, gene flow between monoecious and dioecious populations is limited in regions of geographical overlap between the sexual systems (Dorken *et al.* 2002). It is therefore unlikely that gene flow can explain sex inconstancy in dioecious populations. Second, owing to high selfing rates in inconstant males (Barrett 2003, fig. 6*b*) and strong inbreeding depression (Dorken *et al.* 2002), sex inconstancy should have negative fitness consequences, particularly if there is a cost to seed production. It is therefore unlikely that hermaphrodite alleles would be maintained in dioecious populations without recurrent recombination between sex-determining loci.

In common with many sexually dimorphic species that have originated by the gynodioecy pathway (Lloyd 1976), sex inconstancy in *S. latifolia* is found exclusively among male plants. Our experimental manipulation of resources revealed that approximately one-quarter of male genotypes expressed some level of sex inconstancy, and this proportion increased in the high-fertilizer treatment. By contrast, none of the 27 females in the experiment produced male flowers, and we have never observed female plants producing male flowers in natural populations (M. E. Dorken & S. C. H. Barrett, unpublished data). The absence of sex inconstancy among females of *S. latifolia* contrasts with observations from other dioecious species with monoecious ancestors (Lloyd 1975), but is similar to patterns reported in gynodioecious species (Koelewijn & Van Damme 1996).

(b) *Departures from Mendelian expectations*

We detected two significant departures from the Mendelian expectations of the two-locus model of Charlesworth & Guttman (1999). The first was a small excess of hermaphrodites among the F_1 progeny of crosses between males and plants from monoecious populations. This result could involve differences in the transmission of pollen containing $Su^{F}S^{M}$ versus $Su^{f}S^{m}$ chromosomes (e.g. sex-linked meiotic drive, see Taylor & Ingvarsson 2003). However, for F_2 progenies we detected no departures from Mendelian expectations when F_1 males were used as

parents, a finding inconsistent with segregation distortion of the sex-determining chromosomes. Alternatively, the excess of hermaphrodites could result from the influence of hormones on sex expression, particularly if hormone production is regulated by resource status and plant size (Yin & Quinn 1995; Folke & Delph 1997; Grant 1999). Specifically, if a suppressor of female fertility in males operates through a hormone receptor with a threshold, and the threshold is exceeded in large F_1 progeny, then plants that would otherwise be male may produce a small number of female flowers. Significantly, the observed excess of hermaphrodites was not observed in the first year but was evident only in the second year when plants were much larger. An earlier study demonstrated size-dependent gender modification in monoecious populations of *S. latifolia* (Sarkissian *et al.* 2001) suggesting a link between plant size and the hormones that govern sex expression.

The second departure from Mendelian expectations was the absence of females among the progeny of an inconstant male that was selfed. Assuming the two-locus model, the inconstant male should have the genotype $Su^{f}S^{M}/Su^{f}S^{m}$. It should therefore have produced females upon selfing, as occurred when it was crossed with a female and a male. The absence of females in selfed progeny could be caused by the presence of sex-linked lethal recessive mutations, i.e. linked to $Su^{f}S^{m}$ (D. Charlesworth, personal communication). Such 'X-linked' recessive mutations leading to inviability or infertility occur at low frequencies in species with heteromorphic sex chromosomes, where the absence of recombination between the sex chromosomes exposes these mutations to selection in the hemizygous state (Charlesworth & Guttman 1999). The occurrence of sex-linked lethal recessives in *S. latifolia* is further supported by the absence of female progeny from three full-sib crosses of F_1 plants generated by crossing the inconstant male to a plant from a monoecious population.

(c) *The evolution of dioecy in* Sagittaria latifolia

We have not considered whether dioecy evolved via androdioecy in *S. latifolia*. Because androdioecy is very rare in angiosperms it is thought to be an unlikely intermediate step for the evolution of dioecy (reviewed in Pannell 2002). Moreover, the strongly male-biased floral sex ratios that characterize monoecious populations of *S. latifolia* would make it difficult for males to invade cosexual populations unless they exhibited a large resource-compensation advantage (Sarkissian *et al.* 2001). Nevertheless, since subandrodioecy occurs in the congener *S. lancifolia* (Muenchow 1998), the evolution of dioecy via androdioecy cannot be entirely ruled out. In *S. lancifolia*, males are heterozygous for a dominant suppressor of female fertility, as in *S. latifolia*. The well-studied cases of androdioecy (see Pannell 2002) involve reversions from dioecy (e.g. *Mercurialis annua*, *Datisca glomerata*), and for these species males are also heterozygous. Subandrodioecy in *S. lancifolia* may also represent a reversion from dioecy.

Although there are many examples of the gynodioecy pathway to dioecy (reviewed in Webb 1999), convincing cases of dioecy evolving via the monoecy–paradioecy pathway are surprisingly rare, despite the strong association between monoecy and dioecy among taxonomic groups (Renner & Ricklefs 1995). The main goal of this study was to evaluate which of these pathways is more likely to have been involved in the evolution of dioecy in *S. latifolia*. Our results supporting the Charlesworth & Guttman (1999) model and the occurrence of male but not female inconstancy strongly implicate the gynodioecy pathway. This finding suggests that using ancestral states (e.g. monoecy) to infer that dioecy evolved by one pathway or another may be misleading. The pathways leading to the evolution of dioecy may not be as distinct as previously assumed.

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